Online Supplementary Material

Exercise Restores Dysregulated Gene Expression in a Mouse Model of Arrhythmogenic Cardiomyopathy

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METHODS

Data sharing: The RNA-Seq data is being prepared for submission to GEO (GSE129962). Detailed information about material and methods are available in Online Supplementary Material. All other data and material are available from the corresponding author upon request.

Regulatory approvals. Animal studies were in accord with the NIH Guide for the Care and Use of Laboratory Animals published and approved by the Animal Care and Use Committee (AWC-18-0048).

***Myh6-Cre:Dsp*$^{W/F}$ mice:** The phenotype in the *Myh6-Cre:Dsp*$^{W/F}$ mice has been published and is notable for an age-dependent manifestation of cardiac dysfunction, arrhythmias, excess myocardial fibro-adipocytes, increased apoptosis, and excess mortality. [1] In brief, to determine the genotype, genomic DNA was extracted from mouse tail tissues after digestion in a lysis buffer containing Proteinase K per manufacturer's instructions (cat#158267, QIAGEN Inc, Valencia, CA). Genomic DNA was precipitated using isopropanol at 13,000 rpm for 10 min at room temperature (RT). The DNA pellets were washed in 70% ethanol and rehydrated in water. Approximately 50-100 ng aliquots of DNA were used in the PCR reaction, which was performed using oligonucleotide primers listed in Online Table I. The PCR conditions included an initial denaturation at 94ºC for 5 min followed by 40 cycles of denaturation at 94 ºC for 30 sec, annealing at 58 ºC for 30 sec, and extension at 72ºC for 30 sec. The last cycle was followed by the final extension round for 8 min at 72 ºC. Mice with Cre recombinase-mediated conditional deletion of one copy of the *Dsp* gene under the transcriptional regulation of myosin heavy chain 6 promoter (*Myh6-Cre:Dsp*$^{W/F}$) along with corresponding wild type (WT) littermate controls were used for all the experiments. Sequence of the oligonucleotide primers used for genotyping is provided in Online Table I.

Exercise protocol: Three months old sex-matched WT and *Myh6-Cre:Dsp*$^{W/F}$ mice underwent echocardiography and those with a normal cardiac function were randomized to either regular activity or treadmill exercise. The study design is shown in online Figure I. Those assigned to treadmill exercise were initially trained for 2 days on a five-lane treadmill exercise unit (Touchscreen Treadmill, Panlab, Harvard Apparatus, catalogue # LE8710MTS) and then subjected to a 60-minute treadmill run, starting at 0.2 m/sec at 10% gradient and followed by a stepwise increase in speed and gradient, ending at 0.35 m/sec at 25% gradient (Online Table II). The exercise protocol delivered 5.5 kJ work per day for a 30g mouse (Online Table II). To prevent stopping at the end of the

lane, the unit provided electric current to the shocking grid, which was set at 0.8 mA. In addition, mice were supervised during the exercise and were stimulated manually to ensure continuous exercise.

Isolation of adult mouse cardiac myocytes: Adult mouse cardiac myocytes were isolated as per a published protocol and as reported previously, with minor modifications. [2, 3] Briefly, mice were euthanized, hearts were explanted, and placed in a perfusion buffer containing [120 mM NaCl, 15 mM KCl, 0.6 mM KH2PO4, 0.6 mM Na2HPO4, 1.2 mM MgSO4, 7H2O, 30 mM Taurine, 4.6 mM NaHCO3, 10 mM HEPES, 10 mM 2,3-Butanedione monoxime (BDM), and 5.5 mM Glucose; pH 7.0). After a brief wash in the perfusion buffer, hearts were cannulated retrogradely and perfused with a digestion buffer containing 2.4 mg/ml of Type 2 collagenase (Worthington Cat# LS004176) at a flow rate of 4 ml/min at 42 °C for 3 minutes. Then, 2mM CaCL2 was added and the hearts were digested for a total of 8 min. Upon completion of the enzymatic digestion, the heart was dissected free of vessels and atria, and minced in a stop buffer containing 10% calf serum containing 12.5 µM CaCl2 supplemented with 2 mM ATP. The cell suspension was filtered through a 100 µm cell strainer and myocytes were pelleted by centrifugation at 20 g for 5 minutes. Calcium was reintroduced in a step wise manner by adding 100 µM, 400 µM and 900 µM CaCl2 in the stop buffer and incubating for 4 min in each step followed by centrifugation at 20 g for 4 min. After last step of CaCl2 reintroduction, cardiac myocytes were suspended in a Qiazol reagent (Qiagen Cat# 79306) for subsequent RNA extraction.

RNA Sequencing: Bulk RNA-sequencing (RNA-Seq) was performed (N=5 mice per group), as published. [3, 4] In brief, total RNA was extracted from isolated cardiac myocytes using miRNAeasy mini kit (Cat#217004). RNA concentration was determined using a NanoDrop Spectrophotometer (manufacturer). RNA samples with an RNA Integrity Number (RIN) of > 8 were used to generate strand-specific sequencing libraries after depletion of the rRNA. Sequencing was performed on the Illumina HiSeq 4000 instrument using the paired-end sample preparation chemistry.

Raw RNA sequencing reads were mapped to the mouse reference genome build 10 (UCSCmm10/GRCm38) by Tophat2. [5] Mapped reads were counted using the feature counts. [6] Differentially expressed genes (DEGs) were identified using the edgeR analysis package in R statistical program with the significance level set at $q<0.05$. For comparisons across time points, data was normalized using the Remove Unwanted Variation (RUVr) method, as implemented in the R scientific analysis platform. [7]

Rstudio was used to generate the heat maps and volcano plots using normalized count per million (CPM) values (www.rstudio.com). Circos plots were generated in RStudio using the GO-Chord option.

Pathway analysis: Gene Set Enrichment (GSEA, version 2.2.3, http://software.broadinstitute.org/gsea/) was performed on normalized count per million (CPM) or on ranked gene lists. Ranked lists were created based on the expression levels of DEGs in *Myh6-Cre:Dsp*$^{W/F}$ as compared to WT samples ($q<0.05$). Significance was assessed by analyzing signal-to-noise ratio and gene permutations based on 1,000 permutations. Molecular signature database (MSigDB) 3.0 curated gene sets for hallmark and canonical pathways were used for the analysis. Significant gene sets with enrichment score and a q value cutoff of 0.05 were presented.

Identification of Upstream regulators: Upstream regulators of the dysregulated genes with a q value <0.05 were predicted using Ingenuity pathway analysis software from Qiagen (IPA®, QIAGEN Redwood City). All the upstream regulators with Z-score <-2 and >2 and q <0.05 were reported. For rescue analysis, the status of all upstream regulators that were significantly changed in the *Myh6-Cre:Dsp*$^{W/F}$ were compared to those were not significant in the exercise group or changed in the opposite direction.

Quantitative RT-PCR (qPCR): RNA was extracted from isolated cardiac myocytes using Qiagen miRNeasy Mini Kit (cat # 217006) and treated with DNase 1 to remove the genomic DNA (cat # 79254, QIAGEN). Reverse transcription was performed using approximately 1 μg of total RNA, high capacity cDNA synthesis kit, and random primers (cat# 4368814, Applied Biosystems). Transcript levels of genes of interest were determined by qPCR using specific SYBR Green assays or TaqMan Gene expression assays. Target gene expression levels were normalized to *Gapdh* mRNA levels. Experiments were conducted in duplicates and in at least 5 animals per genotype. Transcript levels were quantified using the ΔCT method and presented as relative (to WT) after normalizing to *Gapdh* values. The list of TaqMan assays and oligonucleotide primers used for qPCR validation are provided in Online supplementary Table I.

Echocardiography: M- and B-mode mouse echocardiography was performed in age- and sex-matched littermates using a Vevo 1100 ultrasound imaging system equipped with a 22-55 MHz MicroScan transducer (MS550D) (FUJIFILM VisualSonics Inc., Toronto, ON, Canada), as published. [3, 4, 8-11] In brief, mice were anesthetized with 1.5% isoflurane and images were obtained in supine position. The leading-edge method was used to measure interventricular septal thickness (IVST), left ventricular end diastolic diameter (LVEDD) and left

ventricular end systolic diameters (LVESD), and posterior wall thickness (PWT) in at least 6 cycles. The mean values of the measurements were used. Indices of cardiac function, including left ventricular fractional shortening (LVFS) and left ventricular mass (LVM) were calculated from the above measurements, the latter using the Devereux formula, as LVM=0.8*[1.04*(ST+LVEDD+PWT)^3-(LVEDD)^3] and was indexed to body weight. [12] The number of mice analyzed by echocardiography in each group is indicated in the corresponding tables.

Electrophysiological (EP) studies: EP studies were performed as published. [13, 14] In brief, mice (N=7 per group) were anesthetized under 2% isoflurane and a 1.1F octapolar electrode catheter (EPR-800, Millar Instruments, Houston, Texas) was inserted via the right jugular vein into the right ventricle. A computer-based data acquisition system (Emka Technologies) was used to record a 6-lead surface electrocardiogram and 4 intracardiac bipolar electrograms. Atria and right ventricle were electrically paced by delivering 2-ms current pulses using an external stimulator (STG-3008, MultiChannel Systems, Reutlingen, Germany). Baseline electrophysiological parameters were determined using standard clinical pacing protocols. Ventricular tachycardia (VT) susceptibility was assessed by overdrive pacing and by delivering single, double, and triple extra-stimuli at the baseline and after intraperitoneal injection with 2 mg/kg isoproterenol (Sigma Aldrich, St. Louis) or 2 mg/kg isoproterenol with 120 mg/kg caffeine (Sigma Aldrich, St. Louis). Each ventricular pacing protocol was repeated twice. Sustained VT was defined as reproducible induced VT episodes of greater than 10 beats in the row.

Gross morphology: The heart was explanted, blood was flushed from the cavities, and weighed. Heart weight corrected for body weight was calculated and the mean values were compared among the groups (N=7 to 13 per group). [8]

Quantification of myocardial fibrosis: Mice (N=4 -7 per group) were euthanized and hearts were rapidly excised and immediately placed in PBS followed by fixing in 10% formalin overnight at 4°C. The hearts were dehydrated in a series of ethanol gradient 70%-100% and washed in xylene followed by embedding in paraffin. Paraffin embedded blocks were sectioned into thin myocardial sections of 5µm thickness using a microtome. The sections were deparaffinized, rehydrated in ethanol gradient, and were stained for Picrosirius Red (Sigma Aldrich, cat# P6744-1GA) following manufacturer's instructions. Collagen volume fraction was calculated as a percentage of the stained area to total myocardial area using ImageJ software (https://imagej.nih.gov/ij/index.html), as published. [3, 9, 10, 14]

Immunoblotting. Immunoblotting was performed as published [3, 15] In brief, aliquots of 10-15 mg of flash frozen ventricular heart tissue was used to extract total myocardial protein. The heart tissue was homogenized in a radioimmunoprecipitation assay (RIPA) buffer (cat #89900, Pierce) containing 25 mM Tris-HCl pH7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.5 % sodium dodecyl sulfate (SDS) in the presence of protease and phosphatase inhibitors (cat #4693116001 and cat #49068459001, Roche, respectively). Protein extracts were quantitated by the Bradford protein assay (Pierce Bradford protein assay kit Thermo Fisher scientific Cat# 23200) using a spectrophotometer set at 595 nm. Approximately 50-100 µg of protein lysates were denatured in a Laemmli sample loading buffer, separated on an SDS-PAGE gel, and transferred to a nitrocellulose membrane. Targeted proteins were detected using specific primary antibodies and the respective horseradish linked secondary antibodies, as listed in Online supplementary Table I.

Immunofluorescence: Expression and localization of the proteins of interest were detected in 5 micrometer thin myocardial frozen sections upon probing with the corresponding primary antibodies and incubation with the secondary antibody conjugates, as published (N=4-5 mice per group). [3, 8, 14] Online supplemental Table I lists antibodies used in immunofluorescence studies.

TUNEL assay: Apoptosis was detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, as published using In-Situ Cell Death Detection Fluorescein Kit (cat# 11684795910, Roche Diagnostics Corporation). [3 1] Briefly 5 µm thick myocardial sections were deparaffinized, rehydrated in ethanol series, and washed in PBS. The sections were boiled in 10 mM sodium citrate (pH 6.0) for 15 min in boiling water, washed in PBS, and were incubated with a TUNEL reaction solution at 37°C overnight containing terminal deoxynucleotidyl transferase and fluroscein-dUTP to label DNA strand breaks. The sections were then incubated in a solution of 1 µg/mL, 4′, 6 Diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma-Aldrich, St Louis, MO; cat# D8417) for 2 min to stain the nuclei followed by mounting the slides in fluorescence mounting media (Dako cat# S3023). The total number of TUNEL positive cells and nuclei were counted in about 8 to 10 fields per each thin section, in at least 5 sections per heart, and in at least 4 animals per group using ImageJ cell counter software (https://imagej.net/Particle_Analysis). A total of 12,000 to 20,000 cells were counted in each heart in at least 4 to 7 mice per group and the percentages of TUNEL positive cells were calculated and compared.

Wheat germ agglutinin (WGA) staining: WGA staining was performed as described previously with minor modifications. [16] In brief, 5 μM thin myocardial sections were deparaffinized, rehydrated, and incubated in boiling 10 mM sodium citrate (pH 6.0) for 20 min. The sections were stained with 1 μg/ml wheat germ agglutinin conjugated to Texas red (Thermo Fisher Scientific, Cat#W21405) followed by incubation in 1 μg/mL DAPI (Sigma-Aldrich, St Louis, MO; cat# D8417). The sections were then mounted with a mounting media (Dako cat# S3023) and examined with a fluorescent microscope (Zeiss, Axioplan Fluorescence Microscope). The number of myocytes in each thin myocardial section was determined upon staining of the sections with an antibody against pericentriolar membrane protein (PCM1), which tags myocyte nuclei in the heart. [17, 18] Images were analyzed by Image J (https://imagej.net), and total pixel counts stained for WGA in each field was determined and subtracted from the total pixel count of the field. The residual pixel count was divided by the number of myocytes in the field, as identified by cells stained positive for PCM1, to determine an average myocyte pixel count. At least 10 fields per section, 5 sections per mice and 4-5 mice per genotype were analyzed, representing about 12,000 to 20,000 cells per each mouse heart.

Secretome analysis: The list of mouse genes coding for secreted proteins (secretome) were obtained from the publicly available curated databases, which list 2,332 genes, whose protein products are very likely to be secreted. [19, 20] DEGs were overlapped with the secretome gene data set to identify those encoding the secretome and assess its enrichment in the *Myh6-Cre:Dsp*$^{W/F}$ myocytes.

Statistical methods: Statistical analyses were performed either using GraphPad Prism 7 (www.graphpad.com) or STATA 10.1 (www.stata.com), and were as published. [3, 9, 14] In brief, data were presented as mean ± SD. Gaussian distribution of the data was determined using Shapiro-Wilk normality test. Normally distributed data were compared using t test between two groups and by ANOVA among multiple groups. The latter was followed by Bonferroni pairwise comparison test to compare differences between two specific groups. Data that departed from normality and non-parametric variables were compared by Mann-Whitney or Kruskal-Wallis test.

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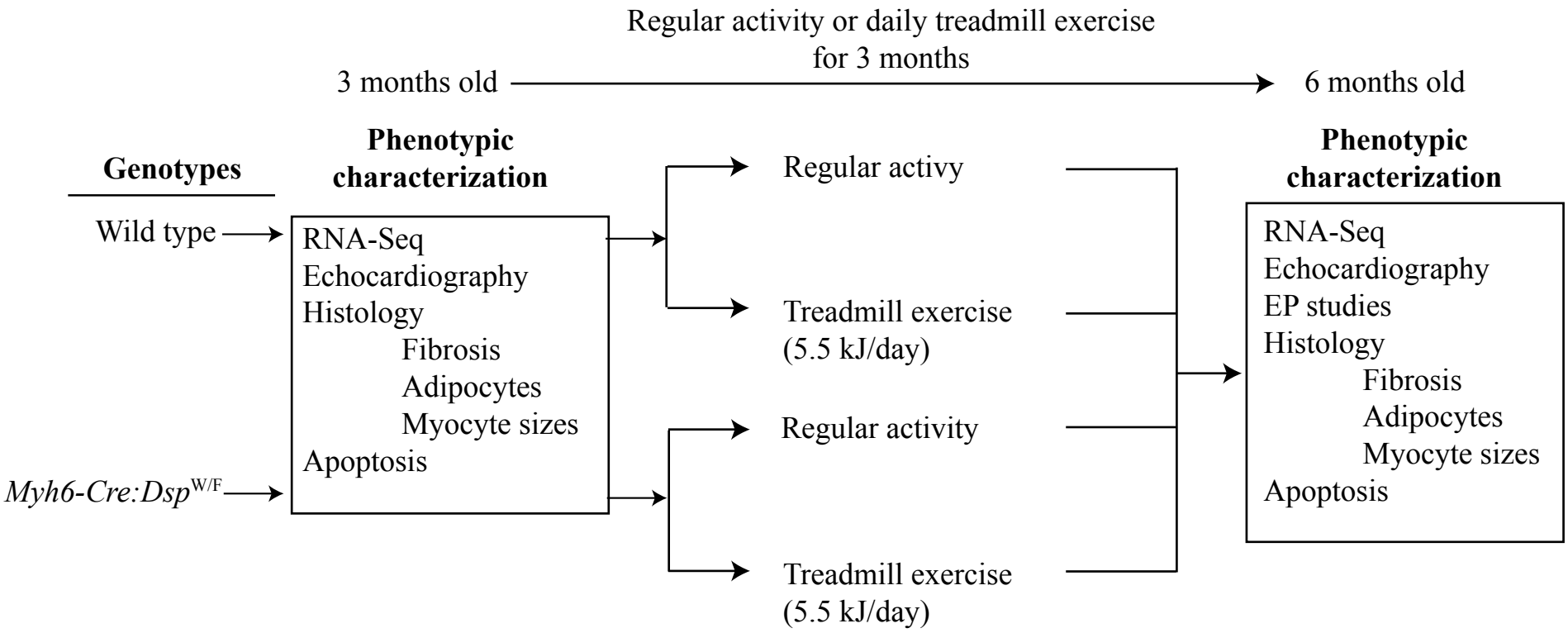
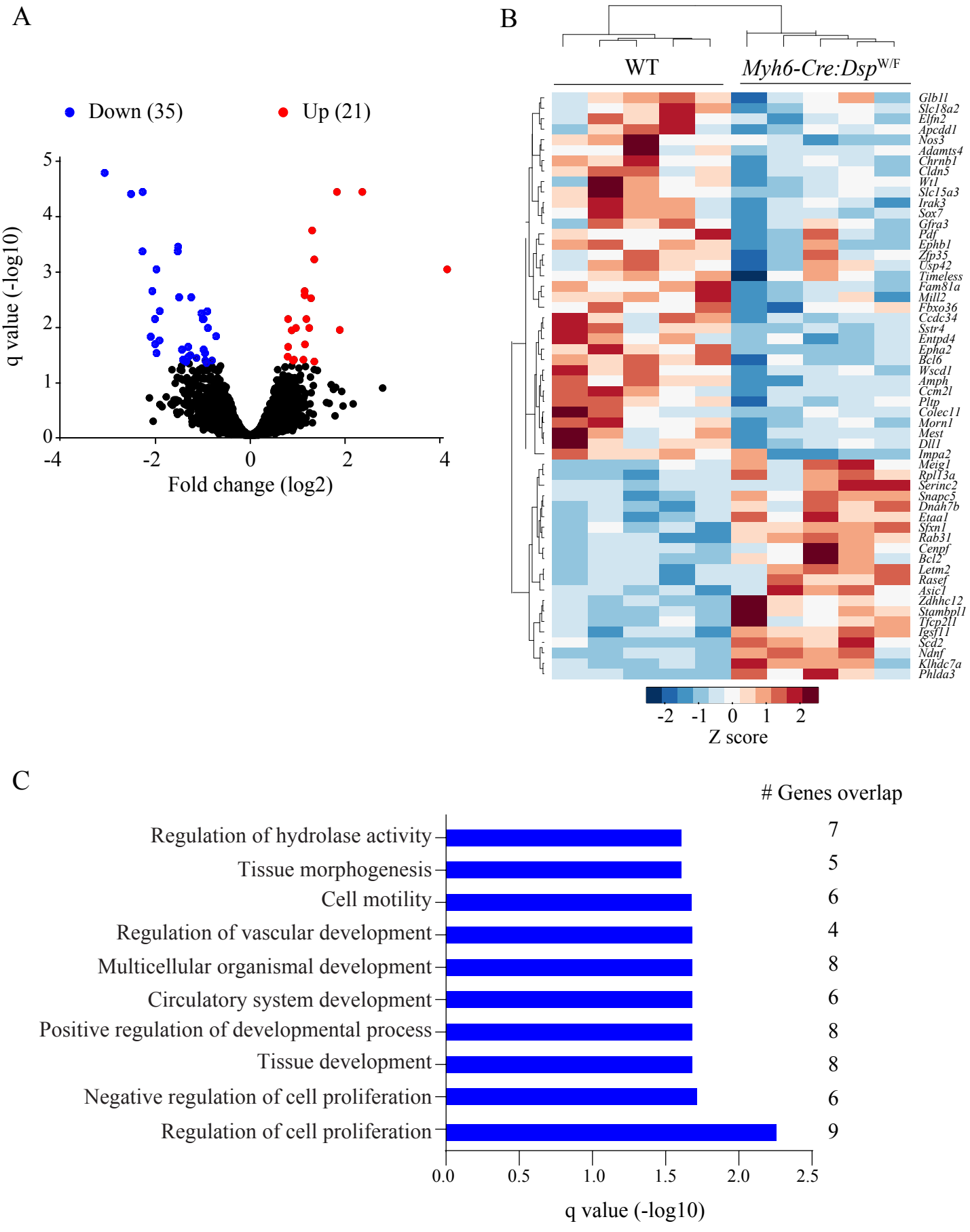
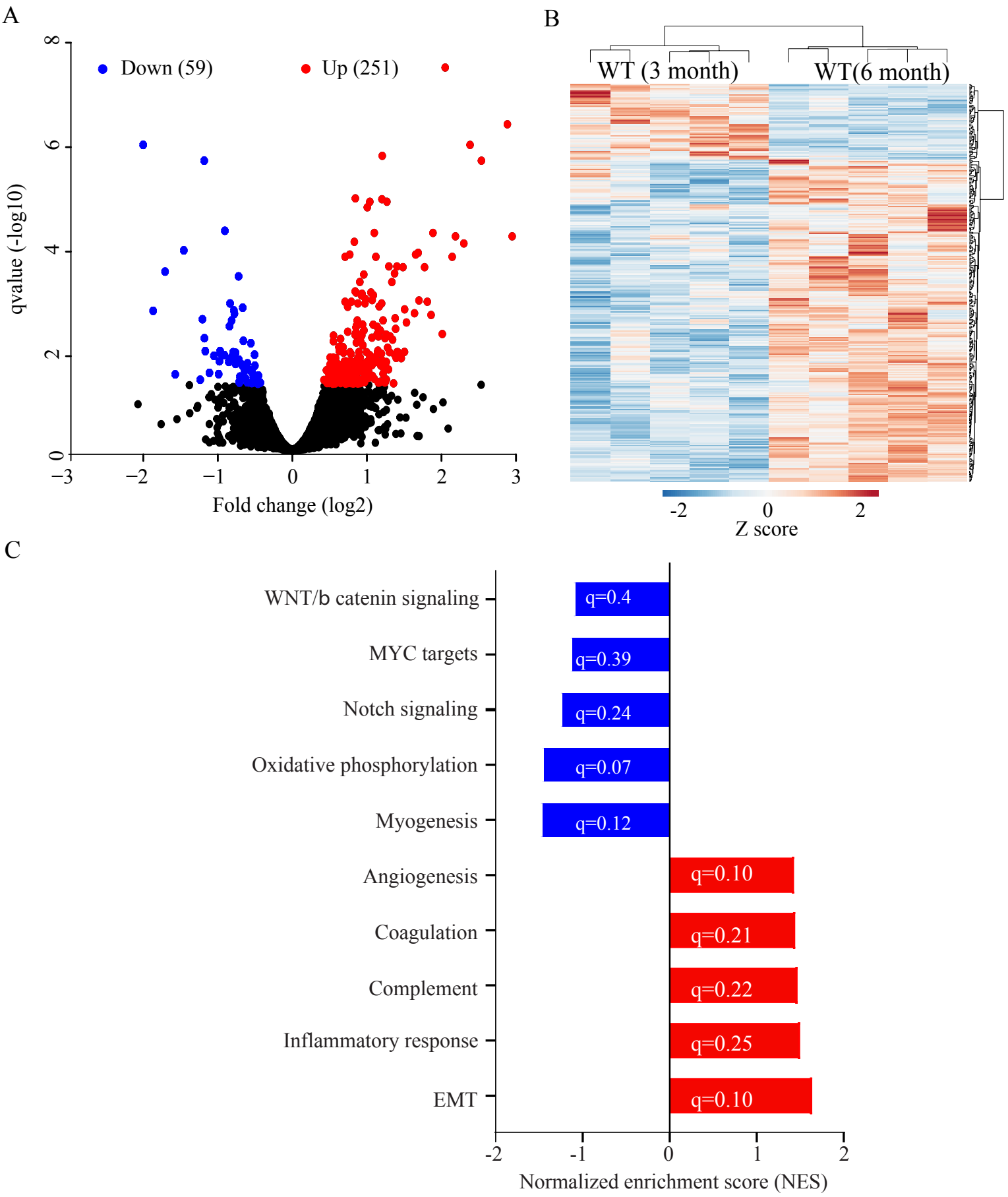


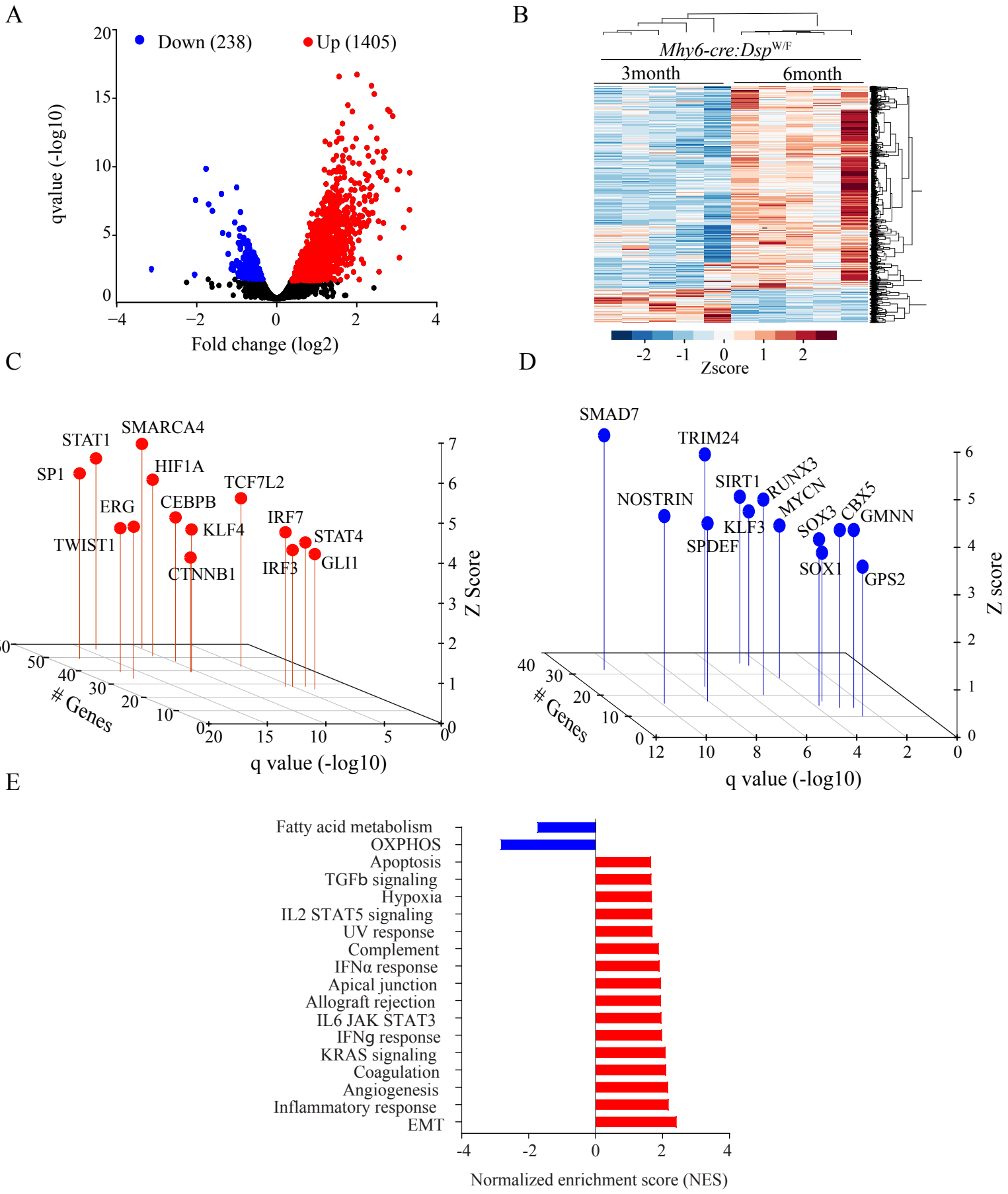
Online Figure 1. Experimental protocol. Three months old wild type and *Myh6-Cre:Dsp*$^{W/F}$ mice were phenotypically characterized at 3 months of age and then randomizd either to regular activity or daily treadmill exercise delivering 5.5 kJ per session for 3 months. Upon completion of 3-month long exercise mice were phenotypically characterized.



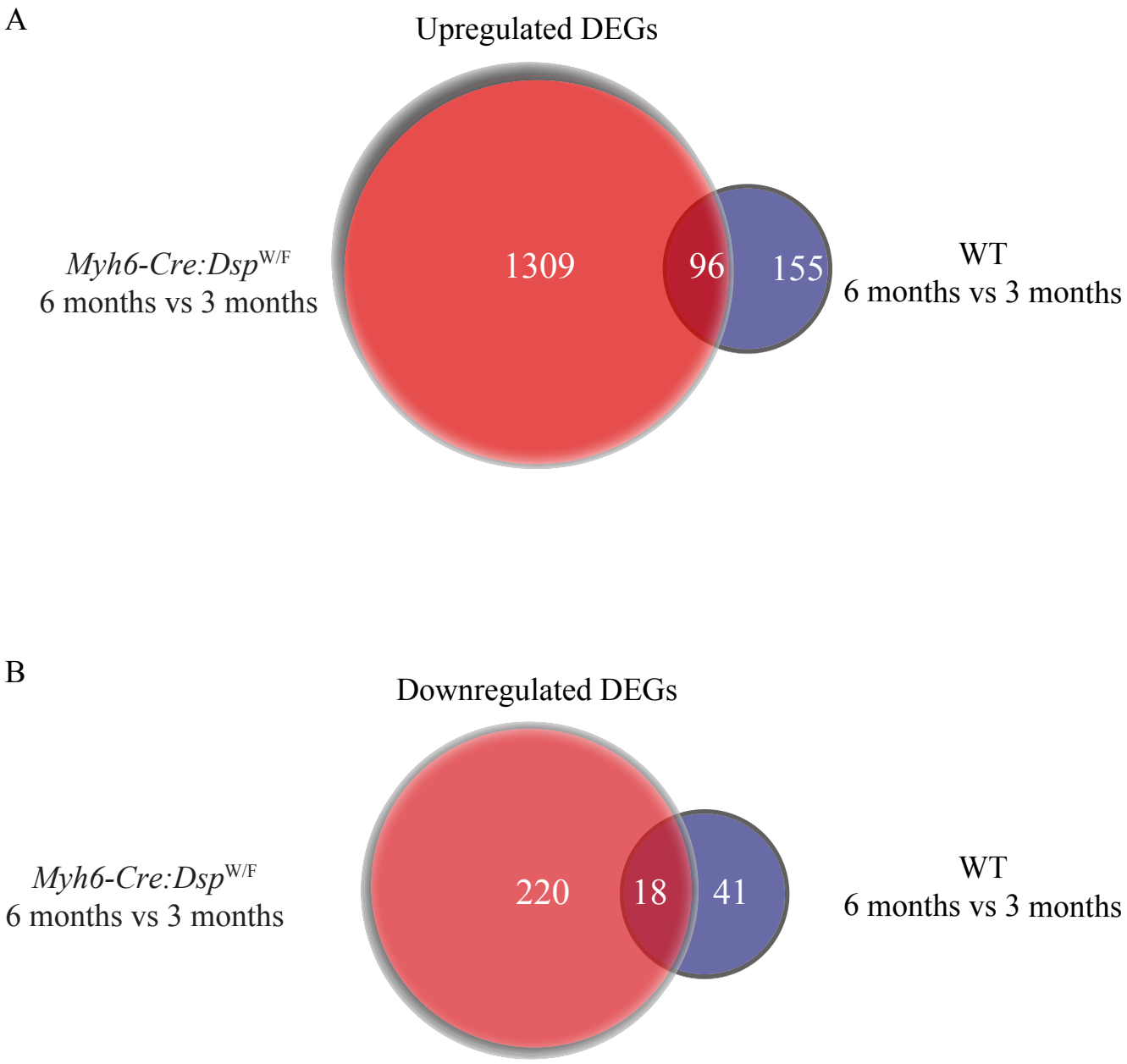
Online Figure II. A. Volcano plot of differentially expressed genes (DEGs) in cardiac myocytes isolated from the hearts of 3-month old *Myh6-Cre:Dsp*$^{W/F}$ mice as compared to the corresponding WT myocytes. **B.** Heat map of DEGs in the same hearts as in panel **A**. Panel **C** shows gene ontology classification of downregulated genes in *Myh6-Cre:Dsp*$^{W/F}$ cardiac myocytes along with the number of genes overlapped with each pathway.



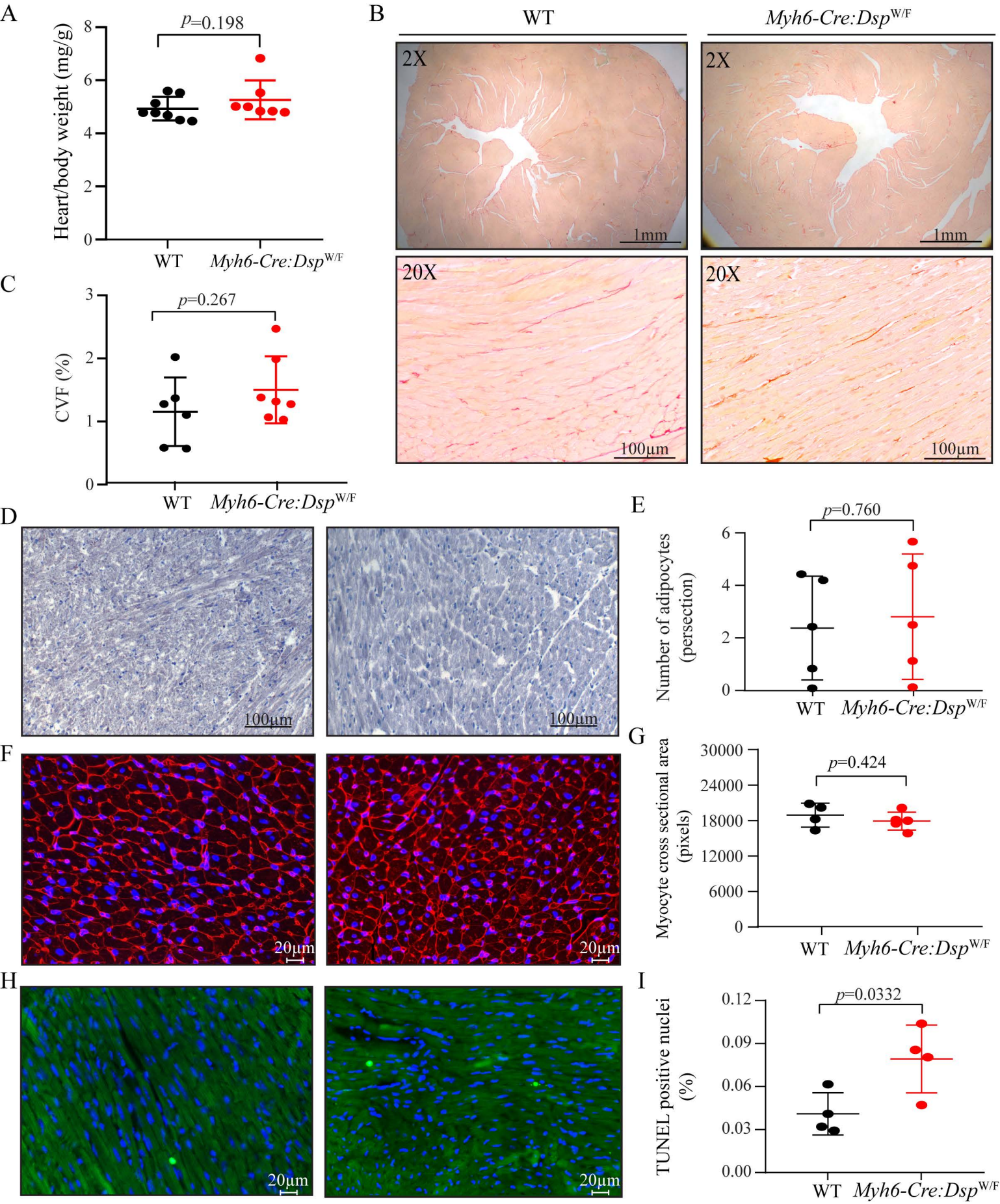
Online Figure III. Temporal evolution of cardiac myocyte transcriptome from 3 to 6 month in wild type (WT) mice.
A. Volcano plot showing upregulated (red) and down-regulated (blue) genes between 3 and 6-months old myocytes.
B. Heat map of differentillay expressed genes (DEGs).
C. Pathways analysis did not identify significantly (FDR<0.05) dysregulated pathways



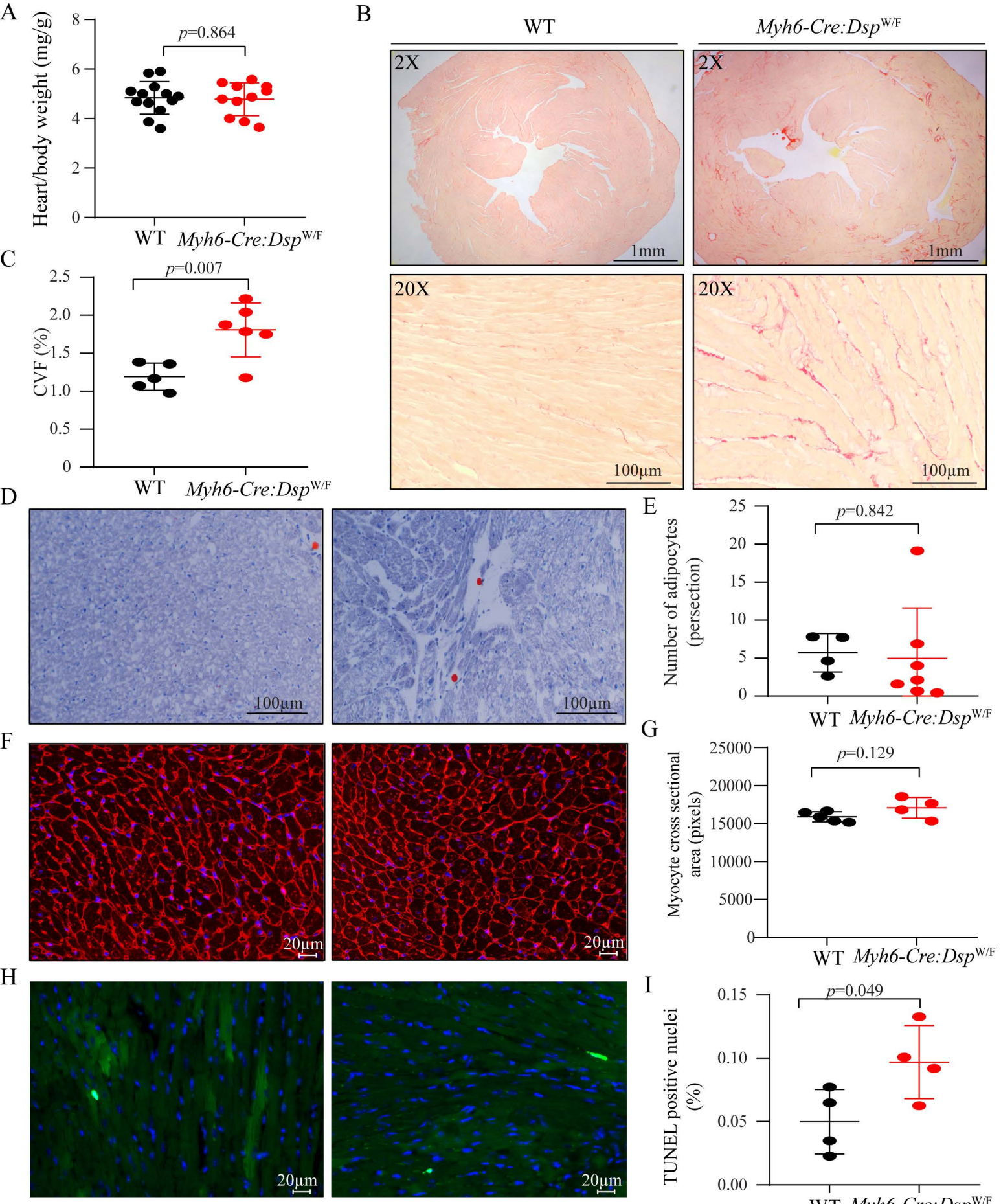
Online Figure IV. Temporal evolution of cardiac myocyte transcriptome from 3 to 6 month in *Myh6-Cre:Dsp*$^{W/F}$ mice. **A.** Volcano plot showing upregulated (red) and down-regulated (blue) genes between 3 and 6 months old myocytes **B.** Heat map of differentially expressed genes (DEGs). IPA showing significantly induced (**C**) and suppressed (**D**) upstream regulators in DEG between between 3 and 6 months old myocytes. **E.** GSEA showing significantly ($q<0.05$) enriched hallmark signatures in the DEG between between 3 and 6 months old myocytes



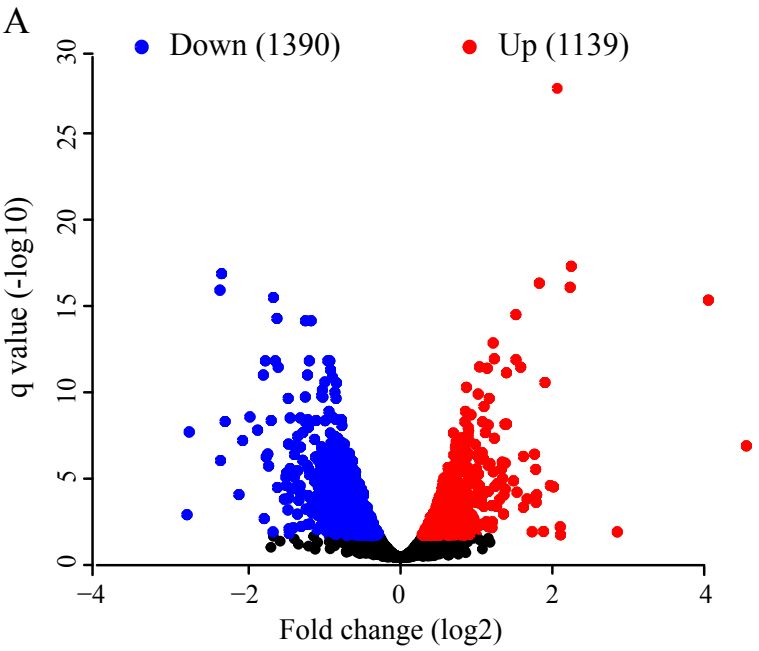
Online Figure V: Genotype-specific transcriptomic evolution in myocytes from 3 to 6 months.
A. Venn diagram of upregulated DEGs showing 96 transcripts overlapped between the two groups, while 1,309 transcripts were specific to *Myh6-Cre:Dsp*$^{W/F}$ myocytes and 155 to WT myocytes.
B. Venn diagram of downregulated DEGs showing 18 transcripts being common to both genotypes, 220 specific to *Myh6-Cre:Dsp*$^{W/F}$ myocytes and 41 to WT myocytes.

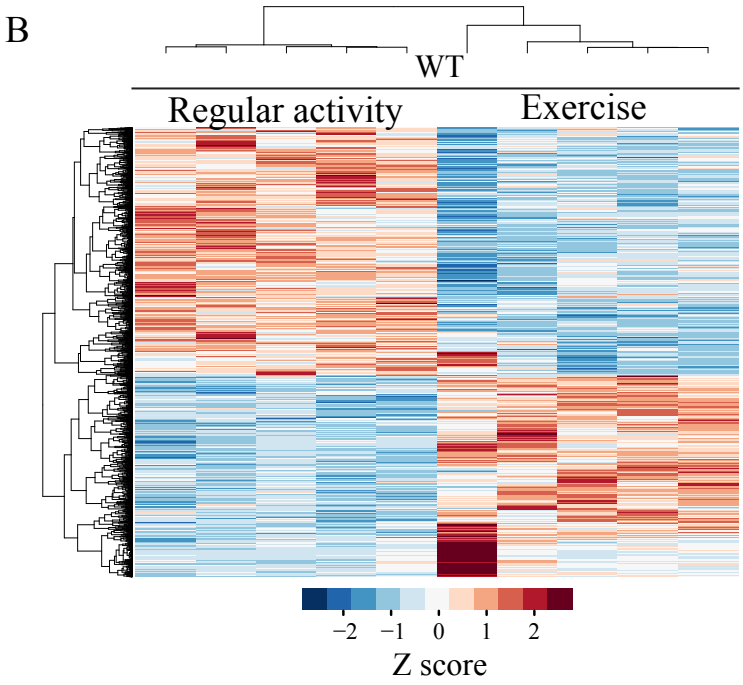


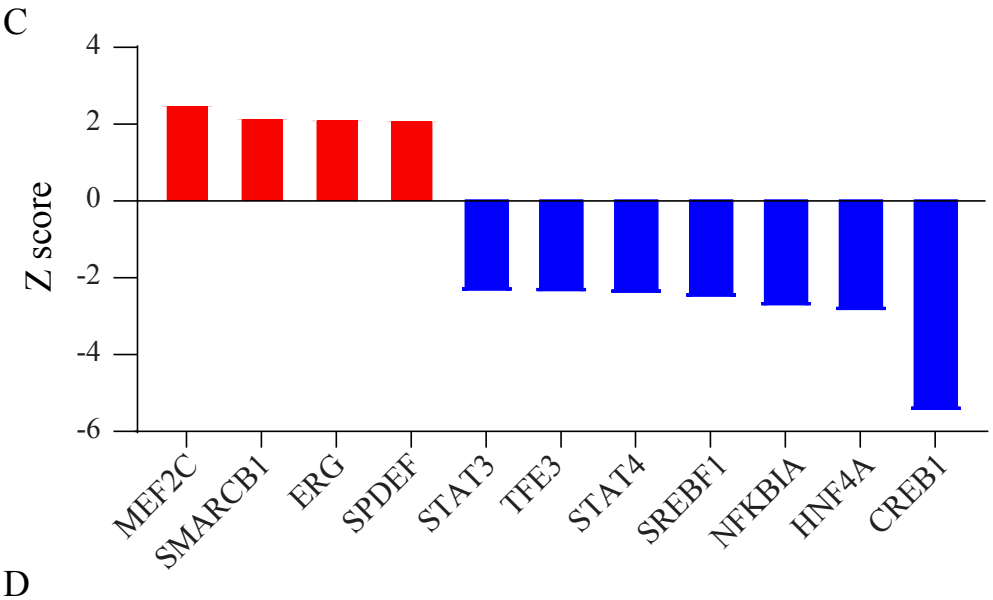
Online Figure VI. Morphological and histological phenotypes in 3 months old wild type (WT) and *Myh6-Cre:Dsp*$^{W/F}$ mice. **A** shows heart weight to body weight ratio; **B**. Picrosirius red stained thin (5 micron) myocardial sections in WT and *Myh6-Cre:Dsp*$^{W/F}$ mice. **C.** Quantitative data on collagen volume fraction (CVF). **D** shows oil red O stained thin myocardial section and **E** the quantitative data of the number of adipocytes per thin section in the two groups. **F** shows wheat germ agglutinin and DAPI co-stained thin myocardial sections. **G.** Mean cross sectional area of myocytes, presented in pixel, corrected for the number of myocytes, identified as PCM1 stained nuclei. **H.** Myocardial cells stained positive for the TUNEL assay along with staining of nuclei with DAPI. **I.** Quantitative number of TUNEL positive cells in the experimental groups.

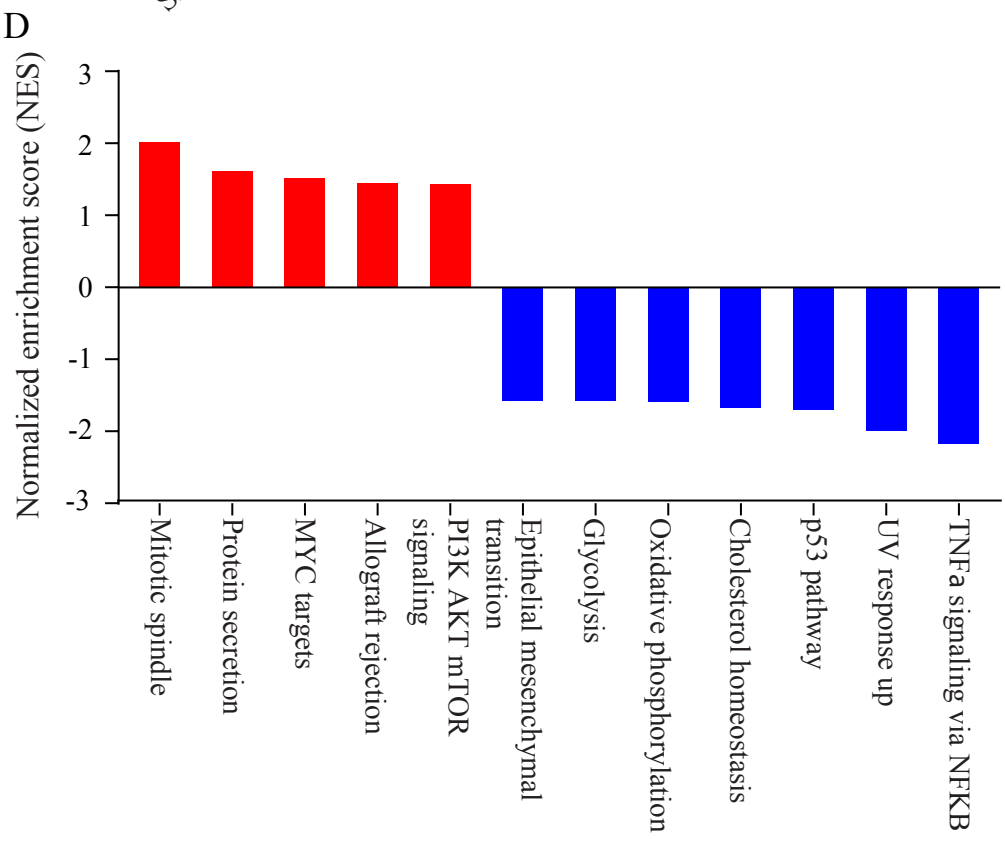


Online Figure VII. Morphological and histological phenotypes in 6 months old wild type (WT) and *Myh6-Cre:Dsp*$^{W/F}$ mice. **A.** Heart weight to body weight ratio. **B**. Picrosirius red stained thin (5 micron) myocardial sections in WT and *Myh6-Cre:Dsp*$^{W/F}$ mice. **C.** Quantitative data on collagen volume fraction (CVF). **D** shows oil red O stained thin myocardial section and **E** the quantitative data of the number of adipocytes per thin section in the two groups. **F** shows wheat germ agglutinin and DAPI co-stained thin myocardial sections. **G.** Mean cross sectional area of myocytes, presented in pixel, corrected for the number of myocytes, identified as PCM1 stained nuclei. **H.** Myocardial cells stained positive for the TUNEL assay along with staining of nuclei with DAPI. **I.** Quantitative number of TUNEL positive cells in the experimental groups









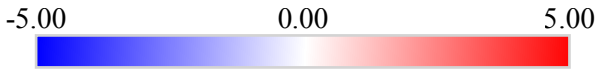
Online Figure VIII: Effects of treadmill exercise on cardiac myocyte gene expression in wild type mice.
A. Volcano plot of differentially expressed genes: Red: increased, Blue: decreased
B. Heat map of differentially expressed genes
C. Dysregulated transcriptional regulators of gene expression, Red: activated, Blue: Suppressed at q<0.05
D. Dysregulated biological pathways; Red: activated; Blue: Suppressed at q<0.05

A

Dsp vs WT	Dsp-Ex vs Dsp	Dsp-Ex vs WT	Dsp-Ex vs WT-Ex	WT-Ex vs WT	
5.0	-4.7		1.7		STAT1
4.0	-2.8			2.1	IRF1
3.9	-5.1		0.6	-1.7	CEBPB
3.9	-4.4				IRF7
3.8	-1.6	1.8	1.3	0.1	TWIST1
3.7	-4.4				IRF3
3.6	-5.8		1.1	-3.7	NFkB
3.5	-2.0	-0.4	1.2		GLI1
3.4	-2.6				SPI1
3.4	-3.7		0.6	-2.3	SMAD4
3.3	-3.5	0.2	0.6	0.4	SP1
3.1	-1.7	-0.5	-0.5	-0.3	CTNNB1
3.1	-4.4			-2.4	STAT4
3.0	-3.7	-0.4			SMAD3
3.0	-1.9				ARNT2
3.0	-1.9				SIM1
3.0	-2.3				MYB
2.9	-1.9	2.6	2.0	2.2	ERG
2.8					NFATC1
2.7			1.5		MTPN
2.7	-1.5		-0.8		KLF4
2.6	-2.4				NCOA2
2.6	-2.9		1.5		SMARCA4
2.5	-3.6	0.7	1.2	-1.7	HTT
2.4			2.0	-1.8	STAT
2.4	-0.9	2.8			TWIST2
2.4			1.5		ETS1
2.4	-2.6				HDAC6
2.4	-1.7		0.2		NFATC2
2.3					FOXM1
2.2		-2.0			PPRC1
2.2	-2.4	-0.1	0.0	-1.6	ATF4
2.2	0.1	1.1	1.4	-0.6	SOX4
2.2					MBD2
2.2	-2.6			-0.7	SMAD2
2.2	-2.2				IRF5
2.2					ETV4
2.2					NFAT
2.0	-0.3			1.0	CCND1
2.0	-4.0	-0.8	1.0	-0.9	HIF1A
2.0	-3.2		-0.6		EGR1
2.0					EBF2

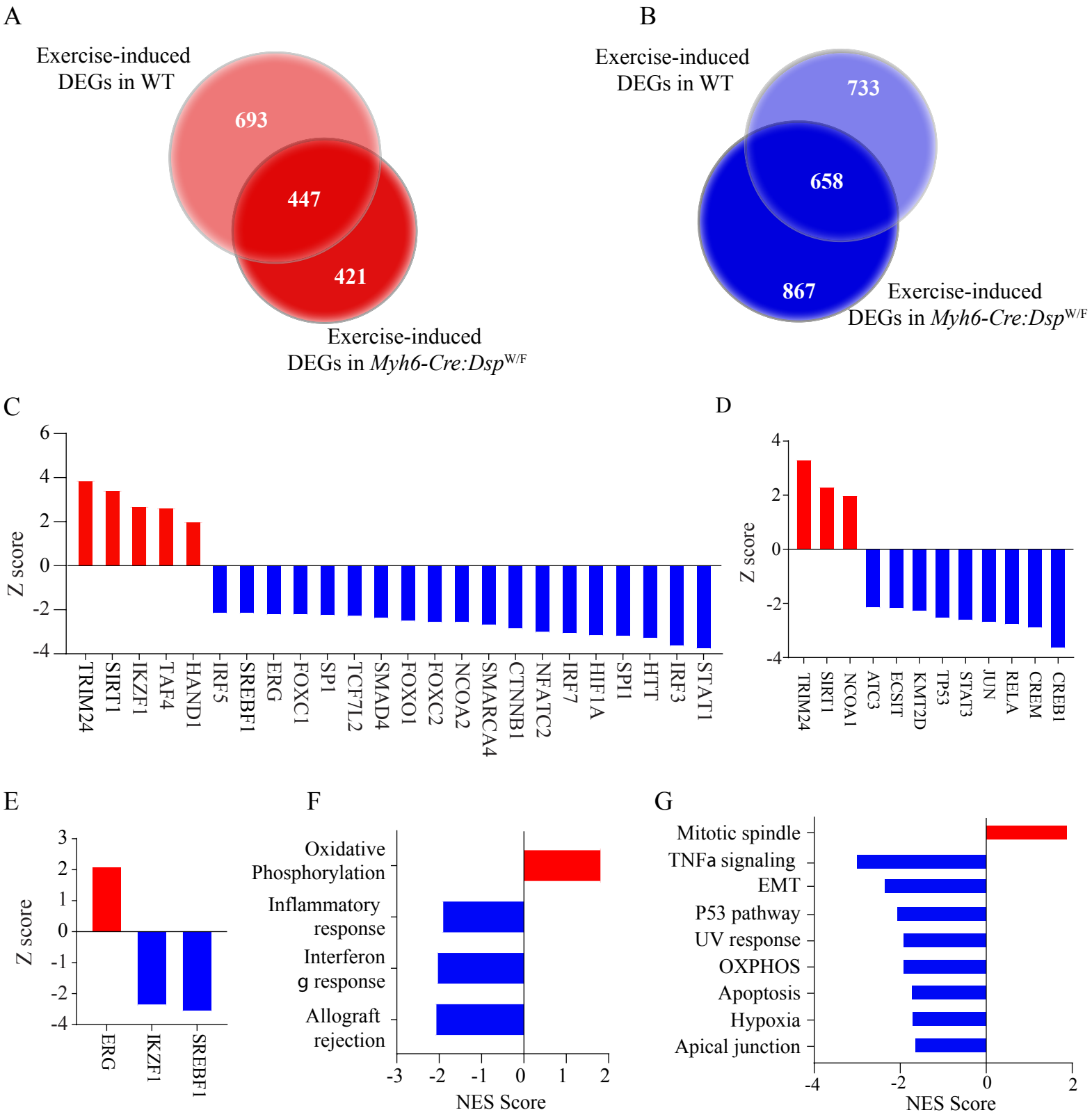
B

Dsp vs WT	Dsp-Ex vs Dsp	Dsp-Ex vs WT	Dsp-Ex vs WT-Ex	WT-Ex vs WT	
-2.0	-0.3	0.3	-1.0	0.4	MYC
-2.1	2.8			2.1	SPDEF
-2.1	0.8		-1.0		HNF1B
-2.1				2.2	ZFP36
-2.1		-2.7	-2.3	-0.9	NUPR1
-2.1	1.3				SOX3
-2.2				-2.2	HLX
-2.2					IRF4
-2.2	3.3	0.5	0.8	2.5	MEF2C
-2.2	2.2	1.8		1.8	HAND2
-2.2					GLIS2
-2.3	1.7		-0.7	1.1	NKX2-3
-2.4					NOSTRIN
-2.5			-1.1		CBX5
-3.0	1.4				RUNX3
-3.4	-0.8			0.3	MYCN
-4.2	5.1			2.5	TRIM24
-4.3	4.1	-0.1	-2.8	1.7	SIRT1
-4.6	1.9		-0.7	1.3	SMAD7



WT: Wild type
WT-Ex- Wild type-Exercise
Dsp: Myh6-Cre:Dsp$^{W/F}$
Dsp-Ex: Myh6-Cre:Dsp$^{W/F-}$ Exercise

Online Figure IX. Effects of exercise on dysregulated canonical transcriptional regulators.
Exercise reverses the dysregulated pathways in *Myh6-Cre:Dsp*$^{W/F}$ myocytes and renders it comparable to those in the wild type myocytes (the first three columns) Additional comparisons are also shown. **A**. Effects on activated (predicted) transcriptional regulators. **B.** Effects on suppressed (predicted) transcriptional regulators



Online Figure X. Genotype-specific effects of exercise on gene expression

A. Upregulated genes in response to exercise exclusive to WT, *Myh6-Cre:Dsp*$^{W/F}$ or common to both

B. Downregulated genes in response to exercise exclusive to WT, *Myh6-Cre:Dsp*$^{W/F}$ or common to both

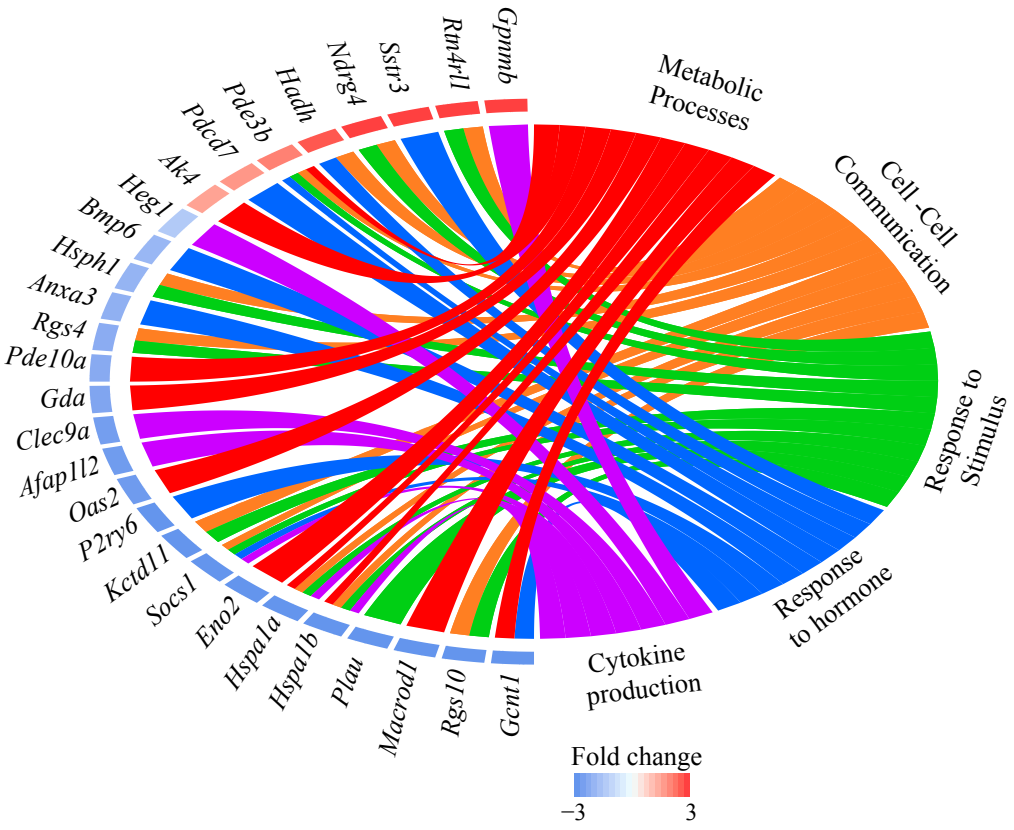
C. Transcriptional regulators enriched in *Myh6-Cre:Dsp*$^{W/F}$-Exercise myocytes only at q<0.05

D. Transcriptional regulators enriched in *Myh6-Cre:Dsp*$^{W/F}$-Exercise and WT-Exercise myocytes at q<0.05

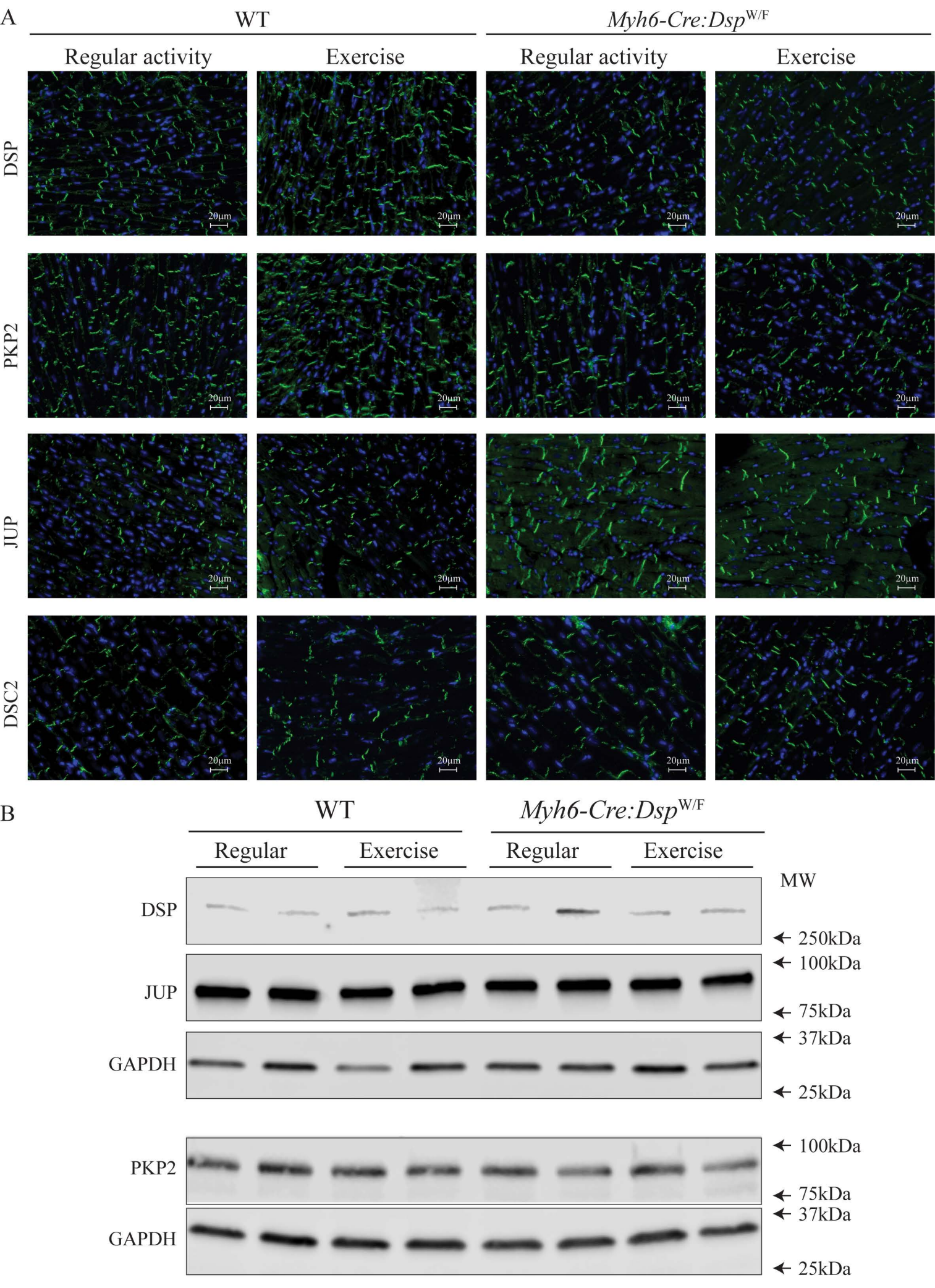
E. Transcriptional regulators enriched in WT-Exercise myocytes only at q<0.05

F. Hallmark biological pathways enriched in *Myh6-Cre:Dsp*$^{W/F}$-Exercise myocytes only q<0.05

G. Hallmark biological pathways enriched in *Myh6-Cre:Dsp*$^{W/F}$-Exercise and WT-Exercise myocytes q<0.05



Online Figure XI. Genotype-by-exercise interactions for the transcript levels. Circos map illustrated genes whose transcript levels were affected by genotype-by-exercise interactions and the involved biological pathways at q<0.05.



Online Figure XII: Panel A shows immunofluoresence panels of myocardial sections stained for selected desmosome proteins in the experimental groups. Panel B shows immunoblots of selected desmosome proteins.

Online Table I

Oligonucleotide Primers, Antibodies, and TaqMan Assays

A. Primers used for genotyping

Transgene	Sequence
Myh6-Cre	Forward: ATGACAGACAGATCCCTCCTATCTCC Reverse: GCGAACCTCATCACTCGTTGCATCGA
$Dsp^{F/F}$	Forward: TAAGCTCCCCTCACTTCTCCAGTC Reverse: TTCTCTTTGTCTGTTGCCATGTGA

B. Antibodies

Antibodies	Concentration	Supplier	Catalogue number
Anti-mouse IgG HRP linked antibody	1:4000 (IB)	Cell Signaling Technology	# 7076
Anti-rabbit IgG HRP linked antibody	1:3000 (IB)	Cell Signaling Technology	# 7074
Donkey anti-Rat IgG, Alexa Fluor 488	1:1000 (IF)	Invitrogen	A21208
Donkey anti-Mouse IgG, Alexa Fluor 488	1:1000 (IF)	Invitrogen	A21202
Donkey anti-Rabbit IgG, Alexa Fluor 594	1:1000 (IF)	Invitrogen	A21207
GAPDH	1:10,000 (IB)	Abcam	ab8245
SFRP1	1:230 (IB)	Abcam	ab4193
TCF7L2	1:5000(IB), 1:200(IF)	Abcam	ab76151
DSP	1:1000(IB), 1:200(IF)	Progen	61003
PKP2	1:1000(IB), 1:50(IF)	Progen	651167
JUP	1:1000(IB), 1:500(IF)	Santa Cruz	sc-1497
DSC2/3	1:200(IF)	Santa Cruz	sc-70994
β-catenin	1:1000(IB), 1:1000(IF)	Santa Cruz	sc-7963
Phospho β-catenin	1:50(IF)	Cell Signaling Technology	#9561

C. Oligonucleotide primers used in qPCR reactions

Name	Sequence
Gapdh	Forward: AACTTTGGCATTGTGGAAGG Reverse: GGATGCAGGGATGATGTTCT
Nr4a1	Forward: CTGCGAAAGTTGGGGGAGT Reverse: CTTGAATACAGGGCATCTCCAG
Tnc	Forward: TGAACGGACTGCCCACATCT Reverse: TTCCGGTTCAGCTTCTGTGGTA
Itgb2	Forward: GTGTCCCAGGAATGCACCAA

	Reverse: GTCCAGTGAAGTTCAGCTTCTG
Vcan	Forward: ACCTGCATGAACCCATCTGC Reverse: GCTCCAGCGATGCTCATGTT
Cyp1B1	Forward: TGTGCCTGCCACTATTACGG Reverse: CTGAACATCCGGGTATCTGGT
P2rx7	Forward: CAGCTGGAACGATGTCTTGC Reverse: CGCTCACCAAAGCAAAGCTAAT
Meox1	Forward: CTGAGCGGCAGGTCAAAGTC Reverse: AGAAGCTGCAGAGTCCCCAT
Cd44	Forward: GGCTCTGATTCTTGCCGTCT Reverse: TCCTGTCTTCCACTGTCCCA
Bgn	Forward: GTCCCTCCCCAGGAACATTG Reverse: GAGCAGCCCATCATCCAAGG
Egfr	Forward: GGGTGGCCTCCTCTTCATAG Reverse: TCCACGAGCTCTCTCTCTTGA
Col4a1	Forward: TTCGCCTCCAGGAACGACTA Reverse: ACAAACCGCACACCTGCTAA
Lox	Forward: ACTGCACACACACAGGGATT Reverse: AGCTGGGGTTTACACTGACC
Igfbp4	Forward: TGCAGACCTCTGACAAGGATG Reverse: GGTGTCCCCACGATCTTCAT
Slit2	Forward: CGGCCTCAGACAAACATCAC Reverse: TGTCATAGCTGGCTCGAACT
Mgp	Forward: GCAACCCTGTGCTACGAATC Reverse: CTTTTGGGCTTTAGCTCGCC
Fbln2	Forward: TCACGCACTACCAGCTCAAT Reverse: CTCATTGCCCTTCGTGATGG
Panx1	Forward: AGATCTCCATCGGTACCCAGA Reverse: GTGGGAGGTTTCCAGACTCG
H2aa	Forward: GAGCAGCTTCAGAGACCTCC Reverse: CTACGTGGTCGGCCTCAATG
C4b	Forward: AGCTCAAAGACTTCCTGATGGAG Reverse: CCTGTAGAGCAGAGCCTCTAA
Irf5	Forward: ACATGTTGCCTTTGACGGAC Reverse: TTCCACTTGCTCCTGGGTAG
Igtp	Forward: TGCTCCTGCCTCTTCTAATCG Reverse: ACTCTCCTTCAGAACCTGCTCA
Postn	Forward: AGAGAAATCCCTGCACGACA Reverse: GTTGGTGCAAACAAGGTCCA
Lrp1	Forward: CAAAGCTGAAGGCTCCGAGT Reverse: TATGCGGACACTCTCATCGC
Mfap5	Forward: ACCTTCCACAGATGACCTAGC Reverse: CCGTAAACTGGTGAAGCAGG
Loxl1	Forward: TATGCCTGCACCTCTCACAC Reverse: TTCACGTGCACCTTGAGGAT
Fbln2	Forward: TCACGCACTACCAGCTCAAT

	Reverse: CTCATTGCCCTTCGTGATGG
Gdf15	Forward: CTCAACGCCGACGAGCTAC Reverse: ACCCCAATCTCACCTCTGGA
H2ab1	Forward: CAGGAGTCAGAAAGGACCTCG Reverse: ACTGGCAGTCAGGAATTCGG
C1qa	Forward: AGGACTGAAGGGCGTGAAAG Reverse: TGGACTCTCCTGGTTGGTGA
Fcgr2b	Forward: GGGAGAAACCCTTCCAGAGG Reverse: GGAGGATTGTATGGGCTGCT
Mrc1	Forward: ATGGATACTGGGCGGACAGA Reverse: CATGCCGTTTCCAGCCTTTC
Apod	Forward: TGCCTGACTATCAAAGGGCA Reverse: TGTCAGTTTCTAACTCTCAGATCA
Igf2	Forward: GGAGATGTCCAGCAACCATCA Reverse: AGTGTGGGACGTGATGGAAC
Atf3	Forward: TCTGCGCTGGAGTCAGTTAC Reverse: GTTTCGACACTTGGCAGCAG
Arc	Forward: CACTCTCCCGTGAAGCCATT Reverse: TCCTCCTCAGCGTCCACATA
Fosb	Forward: TTCTTCTTCTTGGAGGCCGT Reverse: ATCCTCCGGACGAATCGGAA
Col6a3	Forward: GATCGCTTTCGACTCCTCCC Reverse: TGTTTACGTGAACTTCCGTGGT
Bub1	Forward: GCTCTGAAAGCTCCAGGTCA Reverse: GCTGGGCTACAGGCTTAGAT
Nrtn	Forward: CATCCGCATCTACGACCTGG Reverse: GGACACCTCGTCCTCATAGGC
Fkbp5	Forward: TGGTGTTCGTTGTTGGGGAA Reverse: CCAAAACCATAGCGTGGTCC
Rbl2	Forward: AGGAAGAAAACAGAGGCGACA Reverse: CGCACAGCAGAAGATGGTAG
Jph1	Forward: AAGCCAACTCTGGCCCTAAT Reverse: AGGCTATGCTTACCTGTCCTAA
Wee1	Forward: CCGCACACTCCCAAGAGTT Reverse: TGGGGAGTTTGCCGTGTATC
Mad2l1	Forward: CGTGGCCGAGTTTTTCTCATT Reverse: ATGAGCTCGGGGTCAGTAGT
Timp1	Forward: CATGGAAAGCCTCTGTGGATA Reverse: CTCAGAGTACGCCAGGGAAC
Stbd1	Forward: CCAAAGCAGAGCATCTTCGAG Reverse: ACTCTCTGGCATTGACCCAG
Rac2	Forward: GATACCGCAGGTCAGGAGGA Reverse: CGTACCTCAGGGAACCACTTG
IL10ra	Forward: TGCATACGGGACAGAACTGC Reverse: CCGTACTGTTTGAGGGCCAC
Map1a	Forward: GCTGTGAGAGGAAGGTCTGG

	Reverse: GTGCTGCAGTGGGGTTATTT
Nrg1	Forward: CCTTACACTCCACTCCACCG Reverse: AGATGTGGAAAGTTTAGGAGCAGT
Aldob	Forward: TCCACGAGACCCTCTACCAG Reverse: CACCTCCTTGGTCCAACTTGA
Alox5	Forward: ATTGCCATCCAGCTCAACCA Reverse: ACTGGAACGCACCCAGATTT
Selenbp2	Forward: GCCCCTAGTGGTCAAGGGAA Reverse: AAACTGCTTGTCCCAGTCGC
Gsta1	Forward: GCAAGGAAGGCTTTCAAGATTCA Reverse: AGCCAGGATCAACAATTGCTTTTTA
Vwc2	Forward: CCGATCTGCAAAAACGGTCC Reverse: CAATTCTCCACGTGCCCTCT
Acsm5	Forward: CAAGTACCCCAGGAAGGTGG Reverse: CTTCAGTGCATCGCAGCATC
Phlda3F	Forward: GACATGTCAGCTTCTCTGTCC Reverse: GCTGGTTGGCTCCTTCCAT
Mettl11b	Forward: GGTCTCAGGGTACCTGACTG Reverse: GAAGATACAGCCCTCCCGTG
Helt	Forward: AGGGAAAAAGAGCTTTTAGCAGAA Reverse: GTCTTTGGTCTCCATCCGCT
Exoc2	Forward: CCGGCAGGTGTCAGAAACTA Reverse: CTACCACTCTGGCCAAGACC
Cth	Forward: GCACAGTTTGGCCTTTGCAT Reverse: CTGTTGGTGCCTCCATACACT
Polr3g	Forward: CTCCGGAGGTAGAATCTGCG Reverse: GGACCAATGCTGCTAGCCAA

D. TaqMan assays

Gene	**TaqMan Assay ID**
Gapdh	Mm99999915_g1
Col1a1	Mm00801666_g1
Sfrp1	Mm00489161_m1
Igf1	Mm00439560_m1
Mmp2	Mm00439498_m1
Cdkn1a	Ma00432448_m1
Thbs1	Mm00449021_m1
Myh7	Mm0060555_m1
Myoz2	Mm00469639_m1
Fos	Mm00487425_m1

Online Table II Endurance Exercise Protocol				
Stage	**Speed m/s**	**Time (min)**	**Gradient (%)**	**Work (kJ), Weight 30 g**
I	0.2	5	10	0.1764
II	0.2	10	15	0.5292
III	0.2	10	20	0.7056
IV	0.2	10	25	0.882
V	0.25	10	25	1.1025
VI	0.3	10	25	1.323
VII	0.35	5	25	0.77175
		60 min		5.5 kJ

Online Table III

Transcript Levels of Selected Genes Quantified by RNA-Seq and qPCR

WT vs. *Myh6-Cre:Dsp*$^{W/F}$					
RNA-Seq				qPCR	
Gene Symbol	**Fold change**	***p* value**	**Q value**	**Fold change ± SD**	***p* value**
Map1a	4.82	1.08E-05	6.99E-04	6.81±3.79	0.0025
Timp1	6.39	1.99E-11	8.11E-09	4.45±2.89	0.006
Phlda3	2.98	4.38E-11	1.52E-08	2.87±0.78	<0.0001
Stbd1	6.67	2.62E-09	5.48E-07	2.92±0.94	0.001
Nrg1	4.21	4.91E-11	1.58E-08	2.14±0.91	0.04
Il10ra	4.73	1.39E-12	7.43E-10	1.77±0.34	0.008
Rac2	4.89	1.43E-12	7.43E-10	1.47±0.18	0.03
Selenbp2	0.34	4.24E-04	1.17E-02	0.71±0.12	0.004
Cth	0.45	8.55E-08	1.12E-05	0.68±0.17	0.03
Polr3g	0.47	4.73E-09	8.40E-07	0.51±0.07	<0.0001
Alox5	0.28	9.59E-08	1.24E-05	0.43±0.25	0.007
Helt	0.39	3.07E-06	2.34E-04	0.35±0.07	0.0001
Mettl11b	0.37	3.71E-05	1.81E-03	0.34±0.11	0.0002
Exoc2	0.43	9.37E-19	1.39E-15	0.30±0.05	<0.0001
Gsta1	0.49	3.37E-04	9.98E-03	0.24±0.16	0.006
Vwc	0.36	1.88E-07	2.22E-05	0.23±0.08	0.0002
Aldob	0.13	8.64E-22	2.06E-18	0.13±0.09	0.0001
Acsm5	0.36	1.55E-05	9.22E-04	0.20±0.11	0.0003

Online Table IV

Echocardiographic phenotype in 3 months old wild type and *Myh6-Cre:Dsp*$^{W/F}$ mice

	WT	*Myh6-Cre:Dsp*$^{W/F}$	*p value*
N	21	17	N/A
M/F	13/8	10/7	0.555*
Age (days)	98.90±5.64	97.53±3.99	0.458#
Body weight (g)	29.05±3.60	30.37±3.72	0.138
HR (bpm)	510.49±38.67	503.80±38.30	0.299
IVST-d (mm)	0.77±0.09	0.77±0.08	0.463
IVST-s (mm)	1.14±0.16	1.11±0.12	0.301
LVPWT-d (mm)	0.75±0.12	0.70±0.10	0.094
LVPWT-s (mm)	1.08±0.15	1.06±0.17	0.371
LVEDD (mm)	3.63±0.34	3.83±0.45	0.202#
LVEDDI (mm/g)	0.13±0.02	0.13±0.02	0.469
LVESD (mm)	2.31±0.46	2.53±0.35	0.051
EF (%)	66.75±10.89	63.66±7.25	0.161
FS (%)	36.70±8.07	34.29±5.39	0.135
LV Mass (mg)	75.58±11.29	80.09±20.99	0.988#
LVMI (mg/g)	2.63±0.45	2.64±0.55	0.479

Abbreviations: WT: Wild type, *Myh6-Cre:Dsp*$^{W/F}$: Cardiac myocyte specific heterozygous deletion of desmoplakin, M/F: Male/Female, BW: Body weight, g: Grams, HR: Heart rate, bpm: Beats per minute, IVST: Interventricular septum thickness, LVPWT: Left ventricular posterior wall thickness, LVEDD: Left ventricular end diastolic diameter, LVEDDi: LVEDD indexed to the body weight, LVESD: Left ventricular end systolic diameter, FS: Fractional shortening, LVM: Left ventricular mass, LVMI: LVM indexed to the body weight.

* Fisher's exact test
# Kruskal-Wallis test

Online Table V

Echocardiographic phenotype in 6 months old wild type and *Myh6-Cre:Dsp*$^{W/F}$ mice

	WT	*Myh6-Cre:Dsp*$^{W/F}$	*p value*
N	10	10	N/A
M/F	4/6	4/6	1.000*
Age (days)	191.6±15.7	194.60±18.92	0.704
Body weight (g)	31.4±5.0	33.77±6.26	0.424#
HR (bpm)	494.31±40.0	524.73±56.43	0.181
IVST-d (mm)	0.74±0.08	0.67±0.03	0.026
IVST-s (mm)	1.02±0.14	0.94±0.11	0.166
LVPWT-d (mm)	0.84±0.09	0.79±0.11	0.259
LVPWT-s (mm)	1.21±0.09	0.99±0.18	0.004
LVEDD (mm)	3.42±0.14	3.76±0.37	0.016
LVEDDI (mm/g)	0.11±0.02	0.11±0.02	0.755
LVESD (mm)	2.13±0.19	2.68±0.53	0.007
EF (%)	68.43±7.5	56.35±12.12	0.015
FS (%)	37.67±6.0	29.38±7.67	0.015
LV Mass (mg)	72.9±11.9	66.62±15.60	0.280#
LVMI (mg/g)	2.37±0.5	2.01±0.51	0.128

Abbreviations: WT: Wild type, *Myh6-Cre:Dsp*$^{W/F}$: Cardiac myocyte specific heterozygous deletion of desmoplakin, M/F: Male/Female, BW: Body weight, g: Grams, HR: Heart rate, bpm: Beats per minute, IVST: Interventricular septum thickness, LVPWT: Left ventricular posterior wall thickness, LVEDD: Left ventricular end diastolic diameter, LVEDDi: LVEDD indexed to the body weight, LVESD: Left ventricular end systolic diameter, FS: Fractional shortening, LVM: Left ventricular mass, LVMI: LVM indexed to the body weight.

* Fisher's exact test
# Mann-Whitney test

Online Table VI

Transcript Levels of Selected Genes Quantified by RNA-Seq and qPCR

Transcript levels: *Myh6-Cre:Dsp*$^{W/F}$ Regular activity vs. Exercise					
RNA-Seq			qPCR		
Gene Symbol	**Fold change**	***p* value**	**q value**	**Fold change ± SD**	***p* value**
Fkbp5	2.19	2.65E-04	3.56E-03	2.71±0.82	0.002
Myh7	2.29	5.26E-04	5.84E-03	2.10±0.31	0.007
Nrtn	0.40	4.54E-13	2.57E-10	1.88±0.33	0.0002
Wee1	1.70	2.22E-03	1.69E-02	1.84±0.38	0.03
Jph1	1.73	9.44E-09	1.19E-06	1.29±0.23	0.07
Rbl2	2.01	1.70E-09	2.84E-07	1.24±0.15	0.06
Myoz2	1.63	1.15E-05	3.07E-04	0.82±0.17	0.04
Mad2l1	1.55	5.54E-03	3.30E-02	0.87±0.10	0.04
Fosb	0.42	9.74E-09	1.22E-06	0.60±0.23	0.02
Fos	0.43	1.01E-08	1.25E-06	0.47±0.20	0.004
Bub1	0.42	0.000338	0.004215	0.50±0.50	0.001
Thbs1	0.39	7.35E-10	1.40E-07	0.36±0.14	0.003
Col6a3	0.34	6.63E-23	1.97E-19	0.35±0.23	0.004
Atf3	0.32	2.94E-19	7.00E-16	0.24±0.07	0.005
Arc	0.12	4.80E-07	2.91E-05	0.10	0.01

Online Table VII

Transcript Levels of Selected Genes Quantified by RNA-Seq and qPCR

	WT vs. *Myh6-Cre:Dsp*$^{W/F}$ -RA				WT vs. *Myh6 Cre:Dsp*$^{W/F}$ -Ex			
	RNA-Seq		qPCR		RNA-Seq		qPCR	
Gene Symbol	Fold change	q value	Fold change ± SD	*p*	Fold change	q value	Fold change ± SD	*p*
Gdf15	3.57	0.005628	4.20±1.51	0.0014	0.82	0.589	1.02±0.51	0.913
H2-Ab1	3.5	3.56E-08	2.56±0.92	0.014	1.17	0.620	0.96±0.42	0.812
C1qa	2.5	8.4E-07	2.35±0.42	0.004	1.01	0.961	1.07±0.47	0.996
Fcgr2b	3.79	7.17E-08	2.25±0.54	0.07	0.95	0.860	0.42±0.32	0.0588
Igtp	2.54	0.002613	2.26±1.54	0.039	1.31	0.194	0.96±0.20	0.983
H2-Aa	2.98	8.11E-09	2.10±0.67	0.033	1.32	0.187	0.76±0.31	0.451
C4b	2.39	0.000267	1.84±0.26	0.018	1.31	0.194	1.65±0.36	0.0617
Irf5	2.01	0.004069	1.72±0.33	0.0067	ND	NA	0.91±0.28	0.657
Panx1	3.25	1.95E-08	1.69±0.27	0.014	ND	NA	1.34±0.45	0.2797
Mrc1	3.76	8.54E-08	0.83±0.13	0.625	1.99	0.001	1.14±0.38	0.0821
Igfbp4	1.64	8.42E-05	1.93±0.30	0.0004	0.70	0.008	1.10±0.26	0.821
Slit2	1.83	0.033018	2.88±1.05	0.0017	1.11	0.732	1.55±0.65	0.246
Mfap5	2.29	1.12E-05	2.26±0.36	0.0012	1.41	0.175	1.11±0.47	0.893
Lox	2.51	0.000162	2.07±0.88	0.0058	ND	NA	0.99±0.12	>0.999
Postn	2.44	8.11E-09	1.79±0.14	0.0108	1.45	0.020	0.77±0.26	0.272
Mgp	1.81	7.68E-05	1.79±0.30	0.005	1.28	0.160	1.37±0.42	0.2002
Lrp1	1.53	0.00159	1.43±0.18	0.0849	0.95	0.812	0.88±0.32	0.5132
Loxl1	1.53	0.000552	2.07±0.88	0.0194	0.69	0.010	0.99±0.12	0.0067
Fbln2	1.68	0.00058	1.26±0.19	0.247	1.15	0.384	0.86±0.25	0.386

Abbreviations: WT: Wild type; RA: Routine activity; Ex: Exercise; FDR: False discovery rate; SD: Standard deviation; ND: Not detected; NA: not applicable

Online Table VIII

Transcript Levels of the Selected Canonical WNT Targets Quantified by RNA-Seq and qPCR

	WT vs. *Myh6-Cre:Dsp*$^{W/F}$ -RA				WT vs. *Myh6 Cre:Dsp*$^{W/F}$ -Ex			
	RNA-Seq		qPCR		RNA-Seq		qPCR	
Gene	Fold	FDR	Fold change	*p*	Fold	FDR	Fold change	*p*
Tnc	14.49	2.82E-15	7.47±6.14	0.0004	6.34	2.54E-17	2.21±0.99	0.115
Itgb2	3.86	8.11E-09	2.39±1.21	0.02	ND	NA	1.13±0.49	0.977
Sfrp1	1.91	0.040104	2.13±0.60	0.01	1.87	0.0459	1.19±0.54	0.898
Col1a1	2.71	2.37E-17	2.17±0.69	0.035	1.07	0.751051	0.84±0.49	0.452
Igf1	2.20	5.4E-06	2.06±0.65	0.003	2.78	1.68E-07	1.38±0.36	0.246
Vcan	1.82	0.003179	1.98±0.82	0.103	1.39	0.063944	0.72±0.33	0.273
Cyp1b1	3.70	3.51E-11	1.93±1.01	0.129	ND	NA	0.77±0.39	0.579
P2rx7	2.32	4.85E-06	1.76±1.12	0.317	0.90	0.76051	0.81±0.29	0.455
Mmp2	1.78	1.57E-05	1.75±0.72	0.072	1.02	0.90904	0.68±0.27	0.146
Meox1	1.90	0.000487	1.73±0.67	0.083	1.01	0.974719	0.88±0.34	0.689
Cd44	1.58	0.021636	1.70±0.27	0.003	1.90	1.86E-05	1.09±0.20	0.734
Mmp14	2.21	3.56E-08	1.67±0.57	0.111	1.13	0.574955	0.69±0.28	0.168
Bgn	2.02	1.21E-08	1.54±0.60	0.153	1.73	7.14E-05	0.74±0.23	0.250
Egfr	1.86	0.001049	1.51±0.66	0.173	0.74	0.168924	0.74±0.16	0.348
Col4a1	1.53	0.001806	1.32±0.57	0.449	0.82	0.113351	0.72±0.16	0.202
Nr4a1	0.54	0.010357	0.48±0.19	0.028	0.23	1.26E-13	0.13±0.06	<0.0001

Abbreviations: WT: Wild type; RA: Routine activity; Ex: Exercise; FDR: False discovery rate; SD: Standard deviation; ND: Not detected; NA: not applicable

Online Table IX

Effects of Treadmill Exercise on Induction of Cardiac Arrhythmias

			Wild type	*Myh6-Cre:Dsp*$^{W/F}$
N			7	7
M/F			4/3	4/3
Age (months)			6.17±0.03	6.17±0.03
Body weight (g)			27.91±4.30	30.39±4.21
Heart rate (bpm)			508.94±75.72	586.34±83.34
RR interval (msec)			120.49±20.57	104.31±16.30
PR interval (msec)			36.30±3.25	35.83±4.74
QRS duration (msec)			9.54±0.91	8.98±1.41
QT interval (msec)			21.40±2.35	20.00±2.28
SNRT (msec)			105.13±7.65	99.30±9.40
AVERP (msec)			72.14±5.95	68.95±8.64
VT episodes	CL90	N	1	1
		Beats	41	25
	CL70	N	1	2
		Beats	30	14
	CL90/40/40	N	2	0
		Beats	20	0
	CL90/40/30/30	N	7	5
		Beats	104	78
	Burst Pacing 40	N	3	1
		Beats	149	5
	Total	N	14	9
		Beats	344	122

The data of age, body weight, heart rate, RR interval, RR interval, QRS Duration, QT interval, SNRT and AVERP presented with Mean ± SD.

Data after isoproterenol infusion and isoproterenol in combination with caffeine infusion are unremarkable without significant differences between the two groups. Therefore, they are not presented.

Abbreviations: SNRT: sinus node recovery time; AVERP: atrioventricular nodal effective refractory period; VT: ventricular tachycardia; CL: cycle length.